Supplementary Information

Simple and Low-Cost Method for Quantifying Total Phenolic Compounds in Extra Virgin Olive Oils Using Digital Images

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Table S1. Code, expiration and bottling dates, and country of origin for the EVOO samples

EVOO samples code	Country of origin	Expiration date	Bottling date
A1	Portugal	09/27/2024	09/27/2022
A2	Portugal	01/04/2024	07/04/2023
A3	Spain	04/12/2024	12/12/2022
A4	Uruguay	12/12/2024	11/12/2022
A5	Portugal	11/11/2024	12/05/2022
A6	Spain	05/07/2024	11/07/2022
A7	Portugal	05/31/2024	03/20/2022
A8	Portugal	08/25/2024	08/25/2022

EVOO: extra virgin olive oil.

Table S2. 2^4 full factorial design parameters for the colorimetric reaction of the Folin-Ciocalteu method

Factor	Parameter	Low level (−)	Center point	High level (+)
A	Folin-Ciocalteu (FC) volume / mL	0.25	0.63	1
B	Na_2CO_3 volume / mL	2	3	4
C	Na_2CO_3 concentration / % (m/v)	1	5.5	10
D	reaction time / min	30	60	90

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Table S3. Test for evaluation the digital image acquisition based on the LED, distance of capture and RGB channels conditions with responses based on the R^2 of analytical curve of gallic acid

Run	Led	Distance / cm	R^2		
			Red	Green	Blue
1	minimum	10	0.9672	0.7853	0.9649
2	minimum	10	0.9541	0.9173	0.9862
3	maximum	10	0.9430	0.9932	0.7827
4	maximum	10	0.9649	0.9947	0.9264
5	off	13	0.2779	0.3694	0.4477
6	off	13	0.0658	0.0989	0. 3352
7	maximum	16	0.8481	0.9794	0.9797
8	maximum	16	0.8076	0.9547	0.9287
9	minimum	16	0.9309	0.9737	0.9756
10	minimum	16	0.8817	0.9548	0.9624

R^2: coefficient of determination.

Table S4. TPC values, errors, and statistical parameters for analysis of the eight EVOOs using the reference and digital image methods

Sample	Reference method / (mg GAE kg^{-1})	Digital image method / (mg GAE kg^{-1})	Relative error / %	*t*-test	*p*-value	*F*-test
A1	304.69 ± 16.51	340.52 ± 13.30	11.75	–2.11	0.17	1.54
A2	227.70 ± 3.25	236.08 ± 7.91	3.45	–1.27	0.33	0.18
A3	334.74 ± 18.11	358.85 ± 6.64	7.20	–1.95	0.19	7.43
A4	186.38 ± 10.66	185.86 ± 3.58	0.28	0.08	0.94	8.90
A5	378.87 ± 17.59	350.44 ± 7.28	7.50	1.99	0.18	5.84
A6	340.38 ± 11.38	379.59 ± 5.66	11.52	–4.19	0.05	4.04
A7	329.11 ± 7.09	355.57 ± 8.28	8.04	–2.99	0.09	0.73
A8	257.75 ± 12.28	278.09 ± 8.28	7.89	–2.49	0.13	2.20

GAE: gallic acid equivalent.

Table S5. TPC values, errors, and statistical parameters for analysis of the eight EVOOs using the reference and adapted reference methods

Sample	Reference method / (mg GAE kg^{-1})	Adapted reference method / (mg GAE kg^{-1})	Relative error / %	*t*-test	*p*-value	*F*-test
A1	304.69 ± 16.51	326.12 ± 7.74	7.03	–2.43	0.13	4.54
A2	227.70 ± 3.25	218.01 ± 6.17	4.25	2.90	0.10	0.28
A3	334.74 ± 18.11	308.87 ± 11.79	7.71	3.36	0.08	2.31
A4	186.38 ± 10.66	170.85 ± 8.64	8.39	4.18	0.05	1.52
A5	378.87 ± 17.59	403.94 ± 4.23	7.41	–2.41	0.14	13.50
A6	340.38 ± 11.38	328.56 ± 11.13	4.00	1.32	0.32	1.62
A7	329.11 ± 7.09	324.49 ± 12.73	1.50	1.44	0.29	0.34
A8	257.75 ± 12.28	282.79 ± 10.40	9.72	–1.94	0.19	1.39

GAE: gallic acid equivalent.

Table S6. Green analytical procedure index (GAPI) parameters for the three analytical methods used for determination of TPC in EVOOs

Category	Reference method	Adapted reference method	Digital image method
(P1) Sample collection, preservation, transport and storage			
(1) Collection	–	–	–
(2) Preservation	unnecessary	unnecessary	unnecessary
(3) Transport	–	–	–
(4) Storage	store the sample at room temperature and without light	store the sample at room temperature and without light	store the sample at room temperature and without light
(P2) Sample preparation			
(5) Type of method: direct or indirect	indirect	indirect	indirect
(6) Scale of sample preparation	micro-extraction	micro-extraction	micro-extraction
(7) Solvents/reagents used	non-green solvents/reagents used	non-green solvents/reagents used	non-green solvents/reagents used
(8) Additional treatments	none	none	none
(P3) Reagent and solvents			
(9) Amount / mL	10-100	10-100	10-100
(10) Health hazard	no special hazards	no special hazards	no special hazards
(11) Safety hazard	highest NFPA flammability or instability	highest NFPA flammability or instability	highest NFPA flammability or instability
(P4) Instrumentation			
(12) Energy / (kWh *per* sample)	> 1.5	>1.5	≤ 1.5
(13) Occupational hazard	hermetic sealing of analytical process	hermetic sealing of analytical process	emission of vapors to the atmosphere
(14) Waste / mL	10-100	1-10	1-10
(15) Waste treatment	require passivation	require passivation	require passivation
(P5) Quantification			
Central circle	yes	yes	yes

NFPA: National Fire Protection Association.[1]

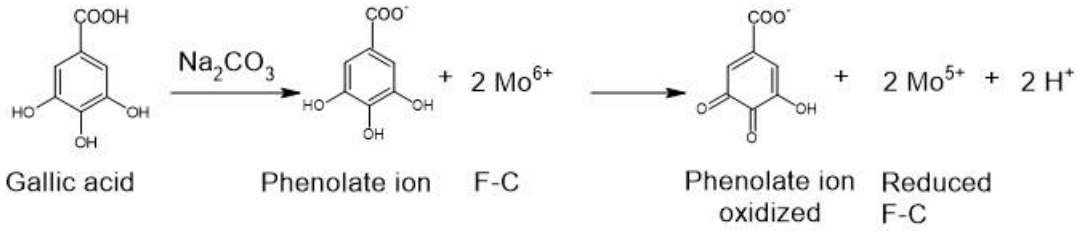

Figure S1. Reaction of phenolic compounds with the Folin-Ciocalteu reagent.

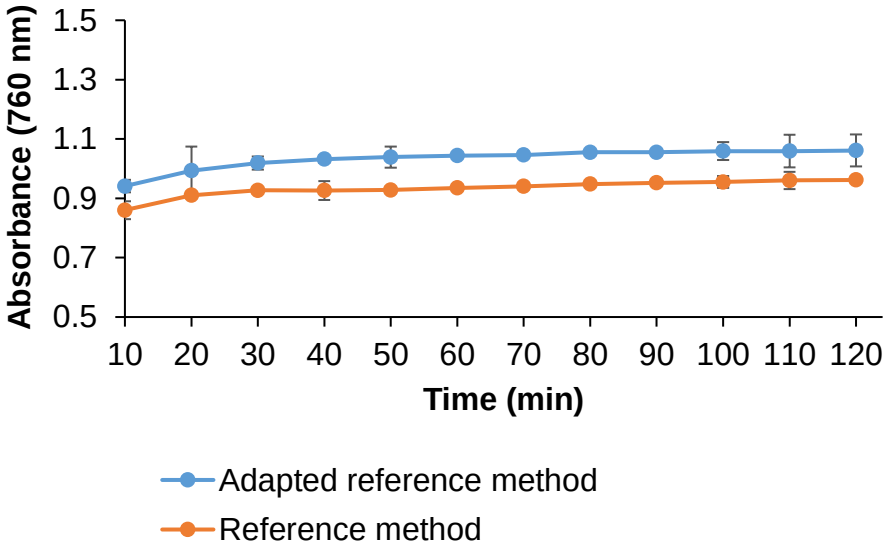

Figure S2. Complex stability of the optimized colorimetric method over time in the interval of 10 to 120 min.

Reference

1. National Fire Protection Association (NFPA); https://www.nfpa.org/ accessed in November 2024.